

## **Arsenic in Urine and Hair by ICP/MS**

### **1 Introduction**

This procedure is used to detect and quantitate arsenic (As) in urine and hair specimens. This procedure does not discriminate between the nontoxic organic forms of arsenic and the toxic inorganic form of arsenic.

### **2 Scope**

This procedure is used to selectively assay arsenic in urine and hair specimens by Inductively Coupled Plasma Mass Spectrometry (ICP/MS) with a collision cell. Urine samples are diluted, spiked with an internal standard and analyzed. Hair samples are first chemically digested then diluted, spiked with an internal standard and analyzed.

### **3 Principle**

Urine specimens are mixed with the Indium Internal Standard Working Solution, diluted up to 10 mL in 2% Nitric Acid in Water Solution and analyzed contemporaneously with a complete matrix matched calibration curve and duplicate sets of matrix matched controls. Hair specimens are massed, completely digested in Tetramethylammonium hydroxide (TMAH), mixed with the Indium Internal Standard Working Solution, diluted up to 10 mL in 2% Nitric Acid in Water Solution and analyzed contemporaneously with a complete urine calibration curve, duplicate sets of controls in urine, a Negative Control Hair sample and duplicate sets of Positive Control Hair samples.

### **4 Specimens**

This procedure is validated for urine and hair. Typically for urine, two 100 µL samples are analyzed. For hair, two 5.5 mg samples are analyzed. However, if it is suspected that the arsenic concentration is above the procedure's linear range, smaller sample sizes or further dilutions of the samples may be analyzed.

### **5 Equipment/Materials/Reagents**

- a. 10 mL and 25 mL volumetric flasks, class A, PMP (VITLAB<sup>®</sup> or equivalent)
- b. 15 mL and 50 mL conical tubes with screw tops, PP (Falcon<sup>®</sup> or equivalent)

- c. 100 mL volumetric flask, class B, PP (Nalgene<sup>®</sup> or equivalent)
- d. 1 L volumetric flask, class B, PP (Nalgene<sup>®</sup> or equivalent)
- e. 25 mL plastic graduated cylinder (Nalgene<sup>®</sup> or equivalent)
- f. Concentrated nitric acid (Optima grade)
- g. Water (Deionized, 18 MΩ)
- h. 2% Nitric Acid in Water Solution (v:v):  
To a 1 L Nalgene<sup>®</sup> volumetric flask, add approximately 800 mL of deionized water. Add 20 mL of Optima grade concentrated nitric acid, fill to the mark with deionized water and mix well. Store at room temperature in plastic. Stable for at least one year.
- i. Tetramethylammonium hydroxide (TMAH), 25% w/w aqueous solution (Electronic grade)
- j. Methanol (HPLC grade or better)
- k. Balance capable of measuring  $\pm 0.1$  mg
- l. Routine laboratory supplies including but not limited to: pipettes, disposable pipettes, forceps, hand shears, etc.
- m. Vortexer
- n. Inductively Coupled Plasma-Mass Spectrometer with a collision cell installed (Thermo-Fisher iCAP Q or equivalent)
- o. Autosampler (CETAC ASX-260 or equivalent)
- p. Cryogrinder (optional)

## 6 Standards and Controls

- a. Indium Internal Standard Stock Solution (1 mg/L in 2% nitric acid solution):  
Purchased from SPEX CertiPrep or an equivalent supplier. Stability and storage determined by manufacturer.
- b. Indium Internal Standard Working Solution (10 µg/L in 2% Nitric Acid in Water Solution):  
To a 100 mL Nalgene<sup>®</sup> volumetric flask that has been washed with 2% Nitric Acid in

Water Solution, add about 80 mL of 2% Nitric Acid in Water Solution. Add 1 mL of Indium Internal Standard Stock Solution, fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.

- c. Arsenic Calibrator Stock Solution (1 mg/L in 2% Nitric Acid in Water Solution): Purchased from SPEX CertiPrep or an equivalent supplier. Stability and storage determined by manufacturer.
- d. Arsenic Calibrator Working Solutions for Urine (10 µg/L – 1000 µg/L):  
The following table shows the preparation of the individual Calibrator Working Solutions for Urine. The Calibrator Working Solutions are prepared in individual 25 mL class A volumetric flasks that have been washed with 2% Nitric Acid in Water Solution. Store at room temperature in plastic. Fill to the mark with 2% Nitric Acid in Water Solution and mix well. Stable for at least one year.

Calibrator Working Solutions for Urine (µg/L)	Volume of Arsenic Calibrator Stock Solution (mL)
10	0.250
25	0.625
50	1.25
100	2.5
250	6.25
500	12.5
800	20.0
1000	25.0

- e. Arsenic Control Stock Solution (100 mg/L in 2% nitric acid solution)  
Purchased from High Purity Standards or an equivalent supplier. Stability and storage determined by manufacturer.
- f. Arsenic Intermediate Control Working Solution (10 mg/L in 2% Nitric Acid in Water Solution):  
To a 25 mL class A volumetric flask that has been washed with 2% Nitric Acid in Water Solution, add about 15 mL of 2% Nitric Acid in Water Solution. Add 2.5 mL of the Arsenic Control Stock Solution (100 mg/L), fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.
- g. Arsenic High Control Working Solution for Urine (800 µg/L in 2% Nitric Acid in Water Solution):  
To a 25 mL class A volumetric flask that has been washed with 2% Nitric Acid in Water Solution, add about 15 mL of 2% Nitric Acid in Water Solution. Add 2.0 mL of the Arsenic Intermediate Control Working Solution (10 mg/L), fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.

- h. Arsenic Low Control Working Solution for Urine (30 µg/L in 2% Nitric Acid in Water Solution):  
To a 25 mL class A volumetric flask that has been washed with 2% Nitric Acid in Water Solution, add about 15 mL of 2% Nitric Acid in Water Solution. Add 75 µL of the Arsenic Intermediate Control Working Solution (10 mg/L), fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.
- i. Arsenic High Control Working Solution for Hair (500 µg/L in 2% Nitric Acid in Water Solution):  
To a 25 mL class A volumetric flask that has been washed with 2% Nitric Acid in Water Solution, add about 15 mL of 2% Nitric Acid in Water Solution. Add 1.25 mL of the Arsenic Intermediate Control Working Solution (10 mg/L), fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.
- j. Arsenic Low Control Working Solution for Hair (50 µg/L in 2% Nitric Acid in Water Solution):  
To a 25 mL class A volumetric flask that has been washed with 2% Nitric Acid in Water Solution, add about 15 mL of 2% Nitric Acid in Water Solution. Add 125 µL of the Arsenic Intermediate Control Working Solution (10 mg/L), fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.
- k. Negative Control Urine:  
Prepared from in-house anonymous donations that are pooled. Collected negative specimens are screened for arsenic. If arsenic is present in an individual specimen, the level must be below 10 µg/L. Combine and stored refrigerated in plastic. Stable for at least one year.
- l. Negative Control Hair:  
Prepared from in-house anonymous donations. Collected negative specimens are screened for arsenic. If arsenic is present, the level must be below 1 ng/mg (50 µg/L). Store at room temperature in paper. Stable indefinitely.

## 7 Sampling

Not applicable.

## 8 Procedure

Appendix 1 contains an abbreviated version of this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

1. Prepare the calibration curve:

To individual 10 mL Vitlab<sup>®</sup> volumetric flasks that have been washed with 2% Nitric Acid in Water Solution add:

- Approximately 5 mL 2% Nitric Acid in Water Solution;
- 100  $\mu$ L of pooled in-house Negative Control Urine;
- 100  $\mu$ L of the appropriate Calibrator Solution (10 – 1000  $\mu$ g/L);
- 100  $\mu$ L of the Indium Internal Standard Working Solution;
- 2% Nitric Acid in Water Solution to the mark, mix well;

Transfer calibrators to labeled 15 mL Falcon<sup>®</sup> tubes.

A zero calibrator/blank is prepared in the same manner except 100  $\mu$ L of deionized water is used instead of 100  $\mu$ L of a calibrator solution.

2. Have a second chemist prepare urine controls:

To individual 10 mL Vitlab<sup>®</sup> volumetric flasks that have been washed with 2% Nitric Acid in Water Solution add:

- Approximately 5 mL 2% Nitric Acid in Water Solution;
- 100  $\mu$ L of pooled in-house Negative Control Urine;
- 100  $\mu$ L of the appropriate Control Solution (30 or 800  $\mu$ g/L);
- 100  $\mu$ L of the Indium Internal Standard Working Solution;
- 2% Nitric Acid in Water Solution to the mark, mix well;

Transfer controls to labeled 15 mL Falcon<sup>®</sup> tubes.

Positive controls are prepared in duplicate.

3. Prepare unknown urine samples:

To individual 10 mL Vitlab<sup>®</sup> volumetric flasks that have been washed with 2% Nitric Acid in Water Solution add:

- Approximately 5 mL 2% Nitric Acid in Water Solution;
- 100  $\mu$ L of unknown sample urine;
- 100  $\mu$ L of deionized water;
- 100  $\mu$ L of the Indium Internal Standard Working Solution;
- 2% Nitric Acid in Water Solution to the mark, mix well;

Transfer calibrators to labeled 15 mL Falcon<sup>®</sup> tubes.

Samples are typically prepared in duplicate.

4. Prepare hair controls (if necessary):

a. To individually labeled 15 mL Falcon<sup>®</sup> tubes:

- Add a minimum of 27.5 mg of negative hair. Accurately record the mass to the nearest 0.1 mg.
- Based upon the recorded mass, add enough TMAH to establish a solution of 5 mg of hair per 100  $\mu$ L of TMAH. (For example, 550  $\mu$ L TMAH is needed for 27.5 mg of hair.)

Allow the hair to completely digest, vortexing occasionally. (This process typically takes at least 8 hours, and the process may be left to proceed overnight.)

b. To individual 10 mL Vitlab<sup>®</sup> volumetric flasks that have been washed with 2% Nitric Acid in water solution add:

- Approximately 5 mL 2% Nitric Acid in Water Solution;
- 100  $\mu$ L of negative hair digest (prepared in 8.4.a above);
- 100  $\mu$ L of deionized water or appropriate Control Working Solution for hair;
- 100  $\mu$ L of the Indium Internal Standard Working Solution;
- 2% Nitric Acid in Water Solution to the mark, mix well;

Transfer controls to labeled 15 mL Falcon<sup>®</sup> tubes.

A minimum of one Negative Control Hair is to be analyzed and the Low and High Hair Controls are to be analyzed in duplicate.

- Note: The low hair control corresponds to 1 ng As per mg hair.
- Note: The high hair control corresponds to 10 ng As per mg hair.

5. Prepare unknown hair samples (if necessary):

a. To individually labeled 15 mL Falcon<sup>®</sup> tubes:

- Add a minimum of 5.5 mg of specimen hair in duplicate. Accurately record the mass to the nearest 0.1 mg.
  - Note: 5.5 mg is a small amount of hair. In order to ensure that a representative hair sample is analyzed, a larger amount of hair may be cut into small snippets and mixed before removing the 5.5 mg sample. Alternatively, a larger hair sample may be cryoground to mix it well.
- Based upon the recorded mass, add enough TMAH to establish a solution of 5 mg of hair per 100  $\mu$ L of TMAH. (110  $\mu$ L TMAH is added to 5.5 mg hair.)

Allow the hair to completely digest, vortexing occasionally. (This process typically takes at least 8 hours, and the process may be left to proceed overnight.)

b. To individual 10 mL Vitlab<sup>®</sup> volumetric flasks that have been washed with 2% Nitric Acid in Water Solution add:

- Approximately 5 mL 2% Nitric Acid in Water Solution;
- 100  $\mu$ L of specimen hair digest prepared in 8.5.a above;
- 100  $\mu$ L of deionized water;
- 100  $\mu$ L of the Indium Internal Standard Working Solution;

- 2% Nitric Acid in Water Solution to the mark, mix well;  
 Transfer controls to labeled 15 mL Falcon® tubes.

6. Analyze calibration samples, control samples and unknown samples by ICP/MS using the instrumental conditions in Section 9 below.

Note: A Negative Control Urine (BLK) should be first in the sequence, followed by the calibrators (STDs), and unknown samples (UNKNOWN) bracketed by Positive Control Urines (QC – LCS). The Negative Control Urine may be reanalyzed as an UNKNOWN between specimens. When hair samples are analyzed, the Negative Control Hair should be analyzed after the urine specimens (as an UNKNOWN) followed by unknown samples (UNKNOWN) bracketed by Positive Control Hair samples (QC – LCS).

## 9 Instrumental Conditions

The following conditions are written to follow Thermo-Fisher's LabBooks software package.

Analytes: As (arsenic) and In (indium)

Acquisition parameters:

Identifier	Dwell time (s)	Channels	Spacing (u)	Measurement mode	Resolution
75As (KED)	0.05	1	0.1	KED	Normal
115In (KED)	0.05	1	0.1	KED	Normal
				# sweeps = 10	

Monitor analytes:

	Uptake	Wash
Minimum	30	30
Maximum	300	300

Survey scan settings:

Start mass (u)	End mass (u)	Dwell Time (s)	Spacing (u)	Resolution	Measurement mode
4.60	245.00	0.01	0.2	Normal	KED

Interference correction: not applicable

Standards: covered in cal/ctrl section

Quantification:

Analyte	Measurement mode	Quantify	Internal Standard	Fit type	Weighting	Forcing	Use for Semi-Quant
75As (KED)	KED	Yes	115In (KED)	Linear	None	Blank	Yes
115In (KED)	KED	No	Use as Internal Standard	Linear	None	Blank	Yes
IS Recovery		Low warning limit: 80%		Low failure limit: 75%			
		High warning limit: 120%		High failure limit: 125%			

Ratios: not applicable

Quality Control:

Calibration Tests / Laboratory Control Standard (LCS)

Analyte	Low failure limit	Low warning limit	High warning limit	High failure limit
75As (KED)	80%	81%	119%	120%

Continuous Tests / Relative Stability Verification (RSV)

Analyte	Verify	Ignore concentration below	unit	Concentration warning limit	Concentration failure limit
75As (KED)	concentration	11	ppb	10%	15%

Autosampler:

Time Settings:	Wash Time (s):	120	Take up Time (s):	45
Rack Settings:	Rack 1 Type:	60-vials (12x5)	Rack 2 Type:	60-vials (12x5)
Autotune Settings:	Autotune rack:	Standard	Autotune vial:	1
Rinse settings	Rinse Rack:	Rinse Station		

## 10 Decision Criteria

### 10.1 Batch Acceptance Criteria

Arsenic should not be detected in the Negative Control Urine specimen above a level of 10 µg/L.

Arsenic should not be detected in the Negative Control Hair specimen above a level of 1 ng/mg.

Arsenic in the Positive Control Urine and Positive Control Hair specimens should quantitate within ±20% of the target value. See the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) for more information.

### 10.2 Sample Acceptance Criteria

The Indium (Internal Standard) response in the unknown specimen(s) should fall within 80% and 120% of the Internal Standard response for the initial blank in the run.

## 11 Calculations

Quantitation is performed by constructing a multi-point calibration curve based on the ratio of the intensity for each calibrator level and the internal standard. The curve is forced through a blank urine specimen with no weighting. See the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) for acceptable practices in calculating quantitative results.

For hair specimens, 5 mg of hair is used in the place of 0.1 mL of urine. Therefore, results as received from the instrument for hair should be divided by 50. For example, if a result of 100 µg/L of As is obtained for a hair specimen, that corresponds to 2 ng As per mg of hair.

When a hair specimen contains arsenic above the method's lower limit of quantitation, it may be analyzed again using a method of standard addition to verify the As concentration.

## 12 Measurement Uncertainty

The critical sources of measurement uncertainty in this procedure include:

- historical random uncertainty of repeated measurements
- accuracy of the pipette used to deliver the sample
- accuracy of the pipette used to deliver the calibrators
- uncertainty in the concentration of the calibration standards
- precision of the delivery of internal standard

When quantitative results are included in an FBI Laboratory report, the measurement uncertainty will be estimated and reported following the *Chemistry Unit Procedures for Estimating Uncertainty in Reported Quantitative Measurements* standard operating procedure (CUQA 13). Information used to derive uncertainty measurements will be tracked in an electronic database.

## 13 Limitations

This method does not discriminate between nontoxic organic forms of arsenic and the toxic inorganic form of arsenic.

- a. Urine - Limit of Detection/Limit of Quantitation (administratively set): 10 µg/L

Urine:	@30 µg/L	@400 µg/L	@800 µg/L
% Bias	2.23	3.20	3.06
% Repeatability	3.06	1.63	2.06
%Intermediate Precision	5.44	2.66	3.07

- b. Hair - Limit of Detection/Limit of Quantitation (administratively set): 1 ng/mg

Hair:	@ 1 ng/mg (50 µg/L)	@ 10 ng/mg (500 µg/L)
% Bias	9.29	11.92
% Repeatability	1.47	2.73
%Intermediate Precision	2.71	3.01

- c. Interferences for urine and hair: no endogenous material/matrices interfered with the analysis of arsenic. For urine, a mixture of nickel, beryllium, cerium, indium, lithium, barium, bismuth, cobalt, lead and uranium at a concentration of 1000 µg/L for each element was analyzed and found not to interfere with the analysis of arsenic.
- d. Carryover: No carryover was observed when a negative control urine specimen was analyzed immediately following a 1000 µg/L calibrator. No carryover was observed when a negative control hair specimen was analyzed immediately following a 10 ng/mg control.
- e. Processed sample stability: For urine, sample stability was evaluated up to seven days after

the initial analysis and remained within  $\pm 20\%$  with refrigerated storage. For hair, sample stability was evaluated up to 24 hours after the initial analysis and remained within  $\pm 25\%$  with refrigerated storage.

## 14 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

## 15 References

*Guidelines for Toxicological Quantitations* (Tox 101); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

*Chemistry Unit Procedures for Estimating Uncertainty in Reported Quantitative Measurements* (CUQA 13); FBI Laboratory Chemistry Unit Quality Assurance and Operations Manual.

FBI Laboratory Chemistry Unit – Instrument Operation and Support Subunit SOP Manual.

*FBI Laboratory Safety Manual*.

Rev. #	Issue Date	History
0	01/08/2016	New document.

**Approval**

Redacted - Signatures on File

**Appendix 1: Abbreviated version of the Procedure for bench use.**

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